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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Paul W. R.	Harris	Auckland, New Zealand
Margaret Anne	Brimble	Auckland, New Zealand

☐ *Additional inventors are being named on the* ______ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Cyclic Proline-Containing Peptide Mimics

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☒ Customer Number 23910 *Type Customer Number here*

OR

☒ Firm *or* Individual Name	Sheldon R. Meyer				
Address	Fliesler Dubb Meyer & Lovejoy LLP				
Address	4 Embarcadero Center, Fourth Floor				
City	San Francisco	State	CA	ZIP	94111-4156
Country	United States	Telephone	415.362.3800	Fax	415.362.2928

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 4

☐ Drawing(s) *Number of Sheets* ____

☐ Application Data Sheet. See 37 CFR 1.76

☐ CD(s), Number ____

☐ Other (specify) ____

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☒ Applicant claims small entity status. See 37 CFR 1.27.

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Respectfully submitted,

SIGNATURE D. Benjamin Borson

Date March 20, 2003

TYPED or PRINTED NAME D. Benjamin Borson

REGISTRATION NO. *(if appropriate)* 42,349

TELEPHONE 415.362.3800

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Attorney Docket No.: NRNZ-1048US0

Synthesis of Cyclic Proline-Containing Peptide Mimics via Ring-Closing Metathesis

Paul W. R. Harris, Margaret A. Brimble* and Peter D. Gluckman

NeuronZ Medicinal Chemistry Group, Department of Chemistry, University of Auckland, 23 Symonds St, Auckland, New Zealand

m.brimble@auckland.ac.nz

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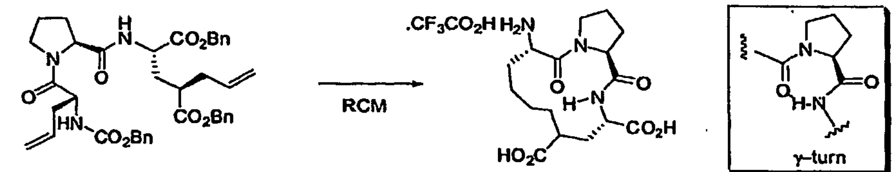
ABSTRACT



89:11, trans:cis 58% *trans only*

Several dienes embedded in di-and tri-peptides which incorporate proline have been prepared and subjected to ring-closing metathesis. Bicyclic peptide mimics of well-defined amide geometry and of varying ring sizes were prepared. Several limitations of the cyclisation step were revealed.

The use of peptidomimetics[1] to mimic the behavior of biologically active peptides is common in the pursuit of a drug candidate. Peptidomimetics are more metabolically stable and protease resistant than peptides and they often adopt well defined conformations.[2]

The use of cyclic peptides provides an elegant approach for the synthesis of peptides of rigid geometry that can be used to probe the bioactive conformation of a given peptide. This strategy can be used to establish the structure of a more potent analogue.[3]

A common method for the synthesis of cyclic peptides[4] relies on construction of an acyclic precursor, typically a diene, that then undergoes cyclisation using Grubbs ring-closing metathesis (RCM).[5] The work reported herein involves the synthesis of dienes from suitable amino acid precursors bearing an allyl group at C(2) or C(5) of proline. RCM of these dienes affords cyclic proline-containing peptides of defined rotamer geometry about the proline amide bond. Several cyclic peptides of varying ring sizes have been prepared and the effect of both stereochemistry and heteroatom location on the metathesis reaction has been investigated.

Synthetic Strategy. We were interested in synthesizing a range of conformationally restricted macrocyclic di- and tri-peptides containing proline, which adopt either *cis* or *trans* stereochemistry about the peptide bond, depending on the substitution on the proline ring.[6] Given that *cis/trans* isomerase enzymes often deliver the bioactive conformation of a peptide,[7] peptidomimetics of defined

[1] For recent advances see (a) Aube, J. *Tetrahedron* 2000, *56*, 9725-9842; (b) Gante, J. *Angew. Chem., Int. Ed. Engl.* 1994, *33*, 1699-1720.

[2] Giannis, A.; Kolter, T., *Angew. Chem., Int. Ed. Engl.* 1993, *32*, 1244-1267.

[3] Marshall, G. R. *Tetrahedron*, 1993, *49*, 3547-3558.

[4] (a) Prabhakaran, E. N.; Rao, I. N.; Boruah, A.; Iqbal, J. *J. Org. Chem.* 2002, *67*, 8247-8250; (b) Banerji, B.; Bhattacharya, M.; Madhu, R. B.; Das, S. K.; Iqbal, J. *Tetrahedron Lett.* 2002, *43*, 6473-6477; (c) Saha, B.; Das, D.; Banerji, B.; Iqbal, J. *Tetrahedron Lett.* 2002, *43*, 6467-6471; (c) Banerji, B.; Mallesham, B.; Kumar, S. K.; Kunmar, A. C.; Iqbal, J. *Tetrahedron Lett.* 2002, *43*, 6479-6483; (d) Belvisi, L; Colombo, L.; Colombo, M.; Di Giacomo, M.; Manzoni, L.; Vodopivec, B.; Scolastico, C. *Tetrahedron* 2001, *57*, 6463-6473; (e) Grossmith, C. E.; Senia, F.; Wagner, J. *Synlett* 1999, 1660-1662; (f) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H: *J. Am. Chem. Soc.* 1996, *118*, 9606-9614; (g) Beal, L. M.; Liu, B.; Chu, W.; Moeller, K. D. *Teterahedron* 2000, *56*, 10113-10125; (h) Hoffmann, T.; Lanig, H.; Waibel, R.; Gmeiner, P. *Angew. Chem., Int. Ed.* 2001, *40*, 3361-3364.

[5] (a) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* 1995, *28*, 446-452; (b) Schuster, M.; Blechert, S. *Angew. Chem., Int. Ed. Engl.* 1997, *36*, 2037-2056; (c) Amstrong, S. K. *J. Chem. Soc., Perkin Trans. 1* 1998, 371-388; (d) Grubbs, R. H.; Chang, S. B. *Tetrahedron* 1998, *54*, 4413-4450; (e) Fürstner, A. *Angew. Chem., Int. Ed. Engl.* 2000, *39*, 3012-3043; (f) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* 2001, *34*, 18-29.

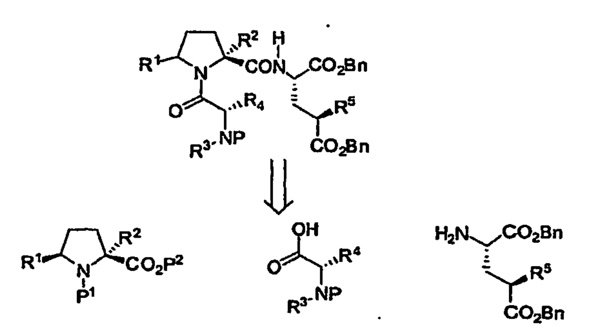
[6] (a) Halab, L.; Lubell, W. D. *J. Peptide Sci.* 2001, *7*, 92-104; (b) Halab, L.; Lubell, W. D. *J. Am. Chem. Soc.* 2002, *124*, 2474-2484.

[7] Fischer, G. *Angew. Chem., Int. Ed. Engl.* 1994, *33*, 1415-1436.

conformation are useful for incorporation into a peptide scaffold.

In order to prepare a series of *cis* and *trans* bicyclic dipeptides incorporating proline, it was decided to introduce an allyl group at either the C(2) or C(5) position. We chose a glycylprolylglutamic acid scaffold onto which were also placed an allyl group on either the glycine or glutamate residue (Scheme 1). Introduction of an allyl group onto the α-carbon and the nitrogen atom of glycine was deemed straightforward. Glutamic acid was chosen in view of the well-established methodology for the stereoselective introduction of an allyl group at C(4).[8]

Scheme 1. General synthesis of diene precursors for RCM based on a glyclyprolylglutamate scaffold.



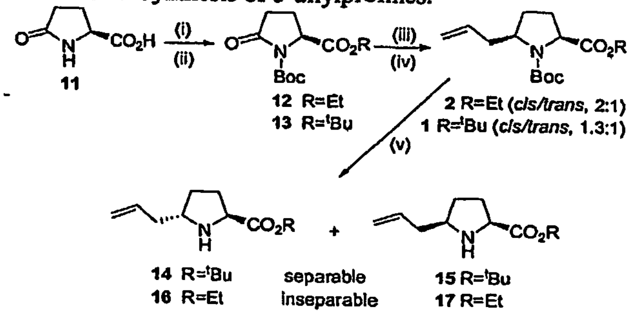
1 R^1 = allyl, R^2 = H, P^1 = Boc, P^2 = ^{t}Bu
2 R^1 = allyl, R^2 = H, P^1 = Boc, P^2 = Et
3 R^1 = H, R^2 = allyl, P^1 = H, P^2 = Me

4 R^3 = allyl, R^4 = H, P = Cbz
5 R^3 = H, R^4 = allyl, P = Cbz
6 R^3 = H, R^4 = allyl, P = Boc
7 R^3 = allyl, R^4 = H, P = Fmoc
8 R^3 = allyl, R^4 = H, P = Boc
9 R^3 = P = allyl, R^4 = H

10 R_3 = allyl

Results and Discussion. The synthesis of proline derivatives bearing an allyl group at C(5) has been reported[9] (Scheme 2). Thus, the ethyl ester[10] and *tert*-butyl ester[11] of readily available (*S*)-pyroglutamic acid **11** were protected as their respective Boc carbamates **12**[12] and **13**.[13] Selective reduction of the lactam carbonyl with $LiEt_3BH$ yielded aminols, which underwent *C*-allylation with allyltributylstannane to afford 5-allylprolines **2**[14] (*cis/trans*, 66:33) and **1**[9] (*cis/trans*, 57:43) in good yield. Neither set of diastereomers could be separated; however, removal of the Boc group with HCl in dioxane allowed small-scale separation of the *trans* and *cis tert*-butyl esters **14** and **15**. The stereochemistry of **14** and **15** was determined using nOe experiments and was supported by literature analogues[9, 10] that suggested that the *cis* isomer **15** would be the major product.

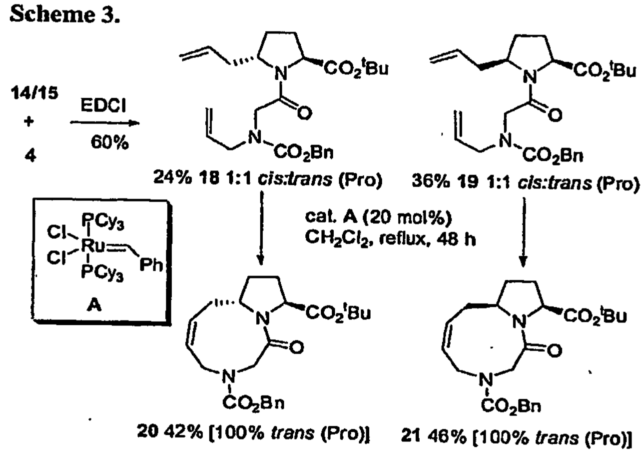
Scheme 2. Synthesis of 5-allylprolines.



(i) for **12**, EtOH/$SOCl_2$, for **13**, 70% $HClO_4$, *tert*-BuOAc (56%);(ii) Boc_2O, DMAP (85% for **2**, 95% for **1**); (iii) $LiEt_3BH$; (iv) for **1**, allyltributylstannane, Me_3SiOTf (70%) for **2**, allyltributylstannane, $BF_3.Et_2O$ (54%); (v) for **1** 4M HCl, dioxane 72% (**14** 31%, **15** 41%); for **2** CF_3CO_2H, *ca.* 80%.

The preparation of (*S*)-2-allylproline methyl ester **3**,[15] *N*-allylglycine carbamates **4**,[16] **7**,[17b] **8**,[17] of *C*-allylglycines[18] **5**[19] and **6**,[20] and of glutamate **10**[8] followed literature procedures or variations thereof.

Scheme 3.



Coupling of *N*-allylglycine derivative **4** with the mixture of diastereoisomeric 5-allylprolines **14** and **15** was effected

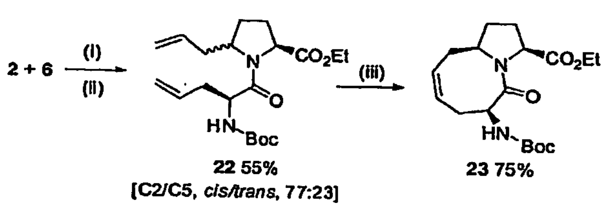
[8] Hanessian, S.; Margarita, R. *Tetrahedron Lett.* 1998, *39*, 5887-5890.
[9] Chiesa, M. V.; Manzoni, L.; Scolastico, C. *Synlett* 1996, 441-443.
[10] Khim, S-K.; Cederstrom E.; Ferri, D. C.; Mariano, P. S. *Tetrahedron* 1996, *52*, 3195-3222.
[11] Besson, M.; Delbecq, F.; Gallezot, P.; Neto, S.; Pinel, C. *Chem. Eur. J.* 2000, *6*, 949-958.
[12] Rahul, J. *Org. Prep. Proceed. Int.* 2001, *33*, 405-410.
[13] August, R. A.; Khan, J. A.; Moody, C. M.; Young, D. W. *J. Chem. Soc. Perkin Trans. 1* 1996, *6*, 506-514.
[14] Mulzer, J.; Schulzchen, F.; Bats, J-W. *Tetrahedron* 2000, *56*, 4289-4298.

[15] (a) Seebach, D.; Boes, M.; Naef, R.; Schweizer, W. B. *J. Am. Chem. Soc.* 1983, *105*, 5390-5398; (b) Wang, H.; Germanas, J. P. *Synlett* 1999, 33-36.
[16] Hon, Y-S.; Chang, R-C. *Heterocycles* 1991, *32*, 1089-1099.
[17] (a) Schleich, S.; Helmchen, G. *Eur. J. Org. Chem.* 1999, 2515-2521; (b) Reichwein, J. F.; Liskamp, R. M. J. *Eur. J. Org. Chem.* 2000, 2335-2344.
[18] (a) Belokon, Y. N.; Taraov, V. I.; Maleev, V. I.; Savel'eva, T. F.; Ryzhov, M. G. *Tetrahedron: Asymmetry* 1998, *9*, 4249-4252; (b) Collet, S.; Bauchat, P.; Danion-Bougot, R.; Danion, D. *Tetrahedron: Asymmetry* 1998, *9*, 2121-2131.
[19] Sun, Z-Y.; Kwon, C-H.; Wurpel, J. N. D. *J. Med. Chem.* 1994, *37*, 2841-2845.
[20] Gao, Y.; Lane-Bell, P.; Vederas, J. C. *J. Org. Chem.* 1998, *63*, 2133-2143.

with EDCI to yield the separable dienes **18** and **19** in moderate yield (60%). The *cis/trans* ratio of the newly created amide (Pro) bond in both these flexible acyclic dipeptides was 1:1. Ring-closing metathesis of the individual 1,10-dienes **18** and **19** was accomplished using Grubbs catalyst A to give the expected cyclononenes **20** and **21** in moderate yield (Scheme 3). The ring closure was not affected by the stereochemistry of the allyl group at C(5) on the proline moiety, both reactions giving similar yields with comparable reaction times and catalyst loadings. Very little deallylation/isomerisation occurred (*cf.* *N*- or *O*-allyl systems).[21] In contrast to their respective precursors, both macrocycles **20** and **21** adopted only the *trans* conformation about the peptide bond.

Subsequent transformations of **20** and **21** proved to be problematic due to the presence of the *tert*-butyl ester and a benzylcarbamate hence our attention was turned to the use of an ethyl ester with a Boc as protecting group. Due to difficulties in separating the ethyl esters **16** and **17**, the mixture of diastereomers **2** was coupled with the C(2)-allylglycine **6** using DCC to afford an inseparable mixture of dipeptides **22** [C(2)/C(5), *cis/trans*, 77:23, *cis/trans* (Pro) ratio undetermined due to complex NMR spectra] in 55% yield (Scheme 4). Subjecting the diene mixture to the standard RCM conditions yielded only one macrocycle **23** in excellent yield (75%). Based on the fact that the *cis* diastereomer was the major component in the precursor, the cyclic metathesis product was assigned as *cis* C(2)/C(5) stereochemistry. Clearly the minor *trans* isomer cyclises extremely slowly relative to the *cis* isomer, a trend that was not observed earlier in the metathesis of the *N*-allyl dienes **18** and **19** (Scheme 3). Gratifyingly cyclooctene **23** adopted only *trans* stereochemistry about the peptide bond.

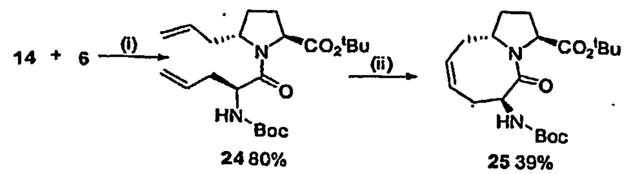
Scheme 4.



(i) CF_3CO_2H; (ii) DCC; (iii) Cat. A (10 mol%), CH_2Cl_2, reflux, 24 h.

In order to access the C2/C5 *trans* diastereomer **25** of **23** it was necessary to use amine **14**; coupling with C(2)-allylglycine **6** using DCC yielded the diene **24** in 80% yield [Scheme 5, *cis:trans* (Pro) ratio undetermined due to complex NMR spectra]. As anticipated ring closure of **24** was extremely slow, required high catalyst loading, and afforded a lower yield of the cyclooctene **25**. However, the peptide bond within macrocycle **25** adopted only a *trans* conformation.

Scheme 5.

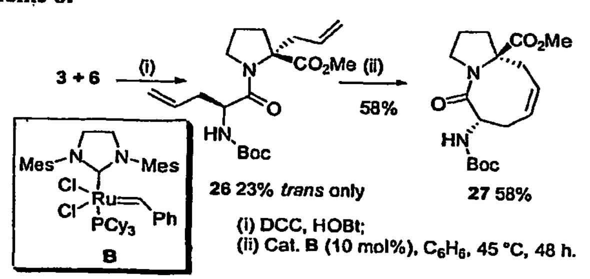


(i) DCC; (ii) Cat. A (30 mol%), CH_2Cl_2, reflux, 116 h.

Grubbs *et al.* [22] have reported that certain *cis* substituted cyclohexane derivatives metathesise under more forcing conditions and in lower yield than the corresponding *trans* isomers. In the present work it was observed that placing the allyl moieties closer together (*C*-allyl vs *N*-allyl) and in certain orientations (*cis* vs *trans*) resulted in significant differences in reactivity.

The above metathesis reactions had involved an allyl group at C(5)on the proline and an allyl unit on the glycine, forming macrocycles containing a *trans* (Pro) amide bond. In order to obtain macrocycles with a *cis* (Pro) amide bond, metathesis between an allyl group at C(2) on proline and an allyl group on the glycine was required. Thus metathesis of **26** with the second generation Grubbs's catalyst (**B**)[23] yielded bicyclic cyclooctene **27**[4h] as a single (*cis*) rotamer in good yield (Scheme 6).

Scheme 6.



(i) DCC, HOBt; (ii) Cat. B (10 mol%), C_6H_6, 45 °C, 48 h.

All attempts to metathesise the corresponding C(2) allyl/*N*-allyl derivatives **28**, **29**, or **30** (Scheme 7) gave complex mixtures probably due to competing deallylation, which is accelerated by the electron-withdrawing Boc, CO_2Bn or Fmoc carbamates. *N,N'*-Diallylglycine[24] derivative **31** was prepared in an attempt to avoid this effect; however, only starting material was recovered after

[21] (a) Cadot, C.; Dalko, P. I.; Cossy, J. *Tetrahedron Lett.* 2002, *43*, 1839-1841; (b) Alcaide, B.; Almendros, P.; Alonso, J. M.; Aly, F. M. *Org. Lett.* 2001, *3*, 3781-3784.

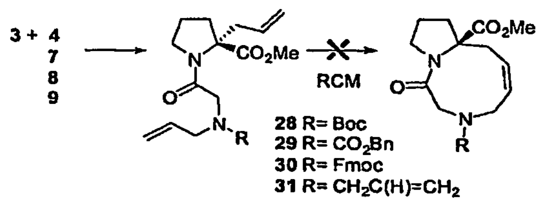
[22] Miller, S. J.; Kim, S-H.; Chen., Z-R.; Grubbs, R. H. *J. Am. Chem. Soc.* 1995, *117*, 2108-2109.

[23] Morgan, J. P.; Grubbs, R. H. *Org. Lett.* 2000, *2*, 3153-3155.

[24] (a) Garro-Helion, F.; Merzouk, A.; Guibe, F. *J. Org. Chem.* 1993, *58*, 6109-6113; (b) Hassner, A.; Maurya, R.; Friedman, O.; Gottlieb, H. E.; Padwa, A.; Austin, D. *J. Org.Chem.* 1993, *58*, 4539-4546.

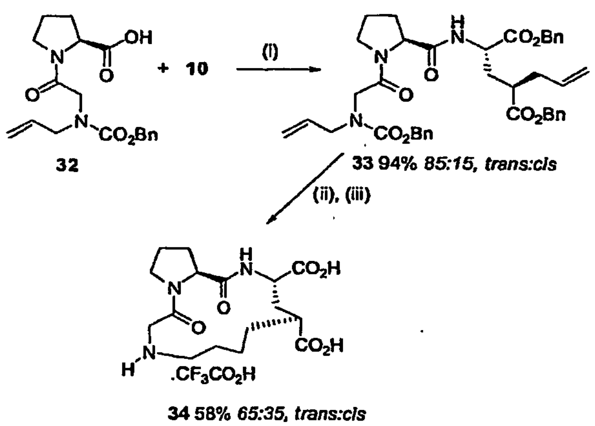
attempted RCM, possibly due to quenching of the catalyst by the reaction with the more basic nitrogen.[25] Conversion of diallylamine **31** into its hydrochloride salt[26] followed by attempted RCM was also unsuccessful.

Scheme 7.



The ring-closing metathesis of dienes contained within a Gly.Pro.Glu scaffold was also examined. The substrate was synthesized (Scheme 8) from coupling **32** with **10** to yield *N*-allylglycyl diene **33**, predominantly as the *trans* (Pro) rotamer. Exposure of **33** to Grubbs catalyst **B** followed by hydrogenation gave macrocycle **34** in 58% yield after purification by HPLC. Cyclotetradecene **34** existed as a 65:35 mixture of *trans:cis* (Pro) rotamers. The increased proportion of the *cis* rotamer may reflect increased flexibility of the Pro amide bond when it is embedded in a larger 14 membered ring.

Scheme 8.

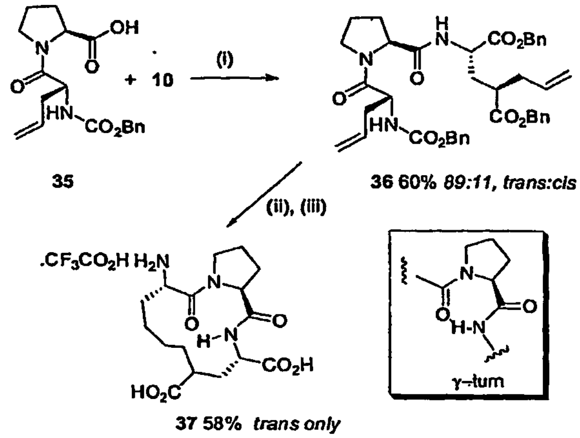


34 58% *65:35, trans:cis*

(i) EtOCOCl; (ii) Cat. **B** (30 mol%), C_6H_6, 45 °C, 65 h; (iii) H_2, 10% Pd/C.

Metathesis of the corresponding *C*-allylglycyl dipeptide **36** and subsequent global deprotection yielded the 13-membered macrocycle **37** (58%) as a single *trans* rotamer (Scheme 9). The observation of only the *trans* amide rotamer may be a consequence of the smaller ring size and/or stabilisation of the structure by the formation of a γ-turn.

Scheme 9.



37 58% *trans only*

(i) EtOCOCl; (ii) Cat. A (20 mol%), CH_2Cl_2, reflux, 48 h; (iii) H_2, 10% Pd/C.

Summary. The synthesis and ring closure of a number of di- and tri-peptides incorporating proline has been accomplished via construction of appropriate diallylated peptide precursors and subsequent olefin metathesis. The proline containing peptides cyclised to afford macrocycles that adopted (except for **34**) a single conformation about the Gly-Pro amide bond. The conformation adopted in each case however was dependant on both the point of attachment of the diene (*C*-allyl vs *N*-allyl) and on the *cis-trans* stereochemistry [C(2) vs C(5)] on the proline ring.

Acknowledgment. The authors thank NeuronZ Ltd for financial support.

Supporting Information Available: Experimental procedures and spectral data for compounds **20**, **23**, and **37**.

[25] Shon, Y-S.; Lee, T. R. *Tetrahedron Lett.* 1997, *38*, 1283-1286.
[26] Fu, G. C.; Nguyen, S. T.; Grubbs, R. H. *J. Am. Chem. Soc.* 1993, *115*, 9856-9857.